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Tomato Leaf DHS cDNA sequence

CGCAGAAACTCGCGGCGCAGTCTTGTTCCGTACATAATCTTGGTCTGCAATAATGGGAGAAGGTCTGAAGTACAGTATCATGGAC **>** E A L G

TCAGTAAGATCGGTAGTTTTCAAAGAATCCGAAAATCTAGAAGGTTCTTGCACTAAAATCGAGGGCTACGACTTCAATAAAGGCGT G LЦ . Ч _ ၁ S 9 ш z ш ш ¥ ــا

TAACTATGCTGAGCTGATCAAGTCCATGGTTTCCACTGGTTTCCAAGCATCTAATCTTGGTGACGCCATTGCAATTGTTAATCAAA NLGDAIAI STGFQAS ELIKSMV

TGCTAGATTGGAGGCTTTCACATGAGCTGCCCACGGAGGATTGCAGTGAAGAAGAAGAGAGATGTTGCATACAGAGAGTCGGTAACC RDVAY ш ш ш E D C S SHELPT 3

TGCAAAATCTTCTTGGGGTTCACTTCAAACCTTGTTTCTTGTTAGAGACACTGTCCGCTACCTTGTTCAGCACCGGATGGT FLGFTSNLVSSGVRDTVRYLVQ TGATGTTGTGGTTACTACAGCTGGTGGTATTGAAGAGGATCTCATAAAGTGCCTCGCACCAACCTACAAGGGGGGACTTCTCTTTAC V V T T A G G I E E D L I K C L A P T Y K CTGGAGCTTCTCCACGATCGAAAGGATTGAACCGTATTGGTAACTTATTGGTTCCTAATGACAACTACTGCAAATTTGAGAATTGG NLLVPNDNYCK SKGLNRIG ATCATCCCAGTTTTTGACCAAATGTATGAGGAGCAGATTAATGAGAAGGTTCTATGGACACCATCTAAAGTCATTGCTCGTCTGGG <u>~</u> ⋖ S ы Н М E K V L E Q I N ш **≻** Σ

FIG.1A



G م ں P V F ж П z Y SYLYWAY لىن 0 z щ

CACTTGGTGACATGCTATACTTCCATTCTTTCAAAAAGGGTGATCCAGATAATCCAGATCTTAATCCTGGTCTAGTCATAGACATT G z о Д Z Ω م Ω . У У YFHS

GTAGGAGATATTAGGGCCATGAATGGTGAAGCTGTCCATGCTGGTTTTGAGGAAGACAGGAATGATTATACTGGGTGGAGGGCTGCC 5 ~ 9 H H E A V ග Z Σ ⋖

TAAGCACCATGTTTGCAATGCCAATATGATGCGCAATGGTGCAGATTTTGCCGTCTTCATTAACACCGCACAAGAGTTTGATGGTA FAVFINTAQ G A D z ∝ Σ Σ ۷ Z

GTGACTCTGGTGCCCGTCCTGATGAAGCTGTATCATGGGGAAAGATACGTGGTGGTGCCAAGACTGTGAAGGTGCATTGTGATGCA × × × -× V ഗ ~ . У G EAVSW 0 م ≃ G

ACCATTGCATTTCCCATATTAGTAGCTGAGACATTTGCAGCTAAGAGTAAGGAATTCTCCCAGATAAGGTGCCAAGTTTGAACATT PILVAETFAAKSKEFSQI

GACTAGTCCTCTTACCATATAGATAATGTATCCTTGTACTATGAGATTTTGGGTGTTTTGATACCAAGGAAAAATGTTTATTTGG GAGGAAGCTGTCCTTCCGACCACACATATGAATTGCTAGCTTTTGAAGCCAACTTGCTAGTGTGCAGCACCATTTATTCTGCAAAA CCATGTTATTTAGTTCTCTTCCTCTTCGAAAGTGAAGAGCTTAGATGTTCATAGGTTTTGAATTATGTTGGAGGTTGGTGATAACT <u> AAAACAATTGGATTTTTAATTTATTTTTTCTTGTT</u>

FIG. 1B





Arabidopsis DeoxyHypusine Synthase (DHS) Predicted Sequence

GAACTCCCAAAACCCTCTACTACTACACTTTCAGATCCAAGGAAATCAATTTTGTCATTCGAGCAACATGG EDDRVFSSVHSTVFKESES GATAAAATCGAAGGATACGATTTCAATCAAGGAGTAGATTACCCAAAGCTTATGCGATCCATGCTCACCAC D K I E G Y D F N Q G V D Y P K L M R S M L T T G F Q A S N L G E A I D V V N O M CAAAAATAAAAATTCCTTCTTTTTGTTTTCCTTTGTTTTTGGGTGAATTAGTAATGACAAAG**AG**TTTGAATT FÉ TGTATTGAAGCTAGATTGGAGACTGGCTGATGAAACTACAGTAGCTGAAGACTGTAGTGAAGAGGAGAAGA V L K L D W R L A D E T T V A E D C S E E ATCCATCGTTTAGAGAGTCTGTCAAGTGTAAAATCTTTCTAGGTTTCACTTCAAATCTTGTTTCATCTGGT P S F R E S V K C K I F L G F T S N L V S S G GTTAGAGATACTATTCGTTATCTTGTTCAGCATCATATGGTTTGTGATTTTTGCTTTATCACCCTGCTTTT V R D T I R Y L V Q H H M TTATAGATGTTAAAATTTTCGAGCTTTAGTTTTGATTTCAATGGTTTTTCTGC**AG**GTTGATGTTATAGTCA D CGACAACTGGTGGTGTTGAGGAAGATCTCATAAAATGCCTTGCACCTACATTTAAAGGTGATTTCTCTCTA TTGGVEEDLIKCLAPTFKGDFSL CCTGGAGCTTATTTAAGGTCAAAGGGATTGAACCGAATTGGGAATTTGCTGGTTCCTAATGATAACTACTG P G A Y L R S K G L N R I G N L L V P N D N Y K F E D W I I P I F D E M L K E Q K E E TCTTGCATCATTGACTTCGTTGGTGAATCCTTCTTTCTCTGGTTTTTCCTTGT**AG**AATGTGTTGTGGACTC N CTTCTAAACTGTTAGCACGGCTGGGAAAAGAAATCAACAATGAGAGTTCATACCTTTATTGGGCATACAAG S K L L A R L G K E I N N E S S Y L Y W A Y GTATCCAAAATTTTAACCTTTTTAGTTTTTTAATCATCCTGTGAGGAACTCGGGGGATTTAAATTTTCCGCT TCTTGTGGTGTTTTGT**AG**ATGAATATTCCAGTATTCTGCCCAGGGTTAACAGATGGCTCTTTGGGGATATG M N I P V F C P G L T D G S L G D M CTGTATTTCACTCTTTTCGTACCTCTGGCCTCATCATCGATGTAGTACAAGGTACTTCTTTTACTCAATA LYFHSFRTSGLIIDVVQ AGTCAGTGTGATAAATATTCCTGCTACATCTAGTGCAGGAATATTGTAACTAGTAGTGCATTGTAGCTTTT CCAATTCAGCAACGGACTTTACTGTAAGTTGATATCTAAAGGTTCAAACGGGAGCTAGGAGAATAGCATAG GGGCATTCTGATTTAGGTTTGGGGCACTGGGTTAAGAGTTAGAGAATAATAATCTTGTTAGTTGTTTATCA AACTCTTTGATGGTTAGTCTCTTGGTAATTTTGAATTTTATCACAGTGTTTATGGTCTTTTGAACCAGTTAAT GTTTTATGAAC**AG**ATATCAGAGCTATGAACGGCGAAGCTGTCCATGCAAATCCTAAAAAGACAGGGATGAT D I R A M N G E A V H A N P K K T G M I AATCCTTGGAGGGGGCTTGCCAAAGCACCACATATGTAATGCCAATATGATGCGCAATGGTGCAGATTACG LGGGLPKHHICNANMMRNGADY CTGTATTTATAAACACCGGGCAAGAATTTGATGGGAGCGACTCGGGTGCACGCCCTGATGAAGCCGTGTCT AVFINTGQEFDGSDSGARPDEA TGGGGTAAAATTAGGGGTTCTĞCTAAAACCĞTTAAGĞTCTGCTTTTTAATTTCTTCACATCCTAATTTATA <u>W G K I R G S</u> A K T V K V C F L I S S H P N L TCTCACTCAGTGGTTTTGAGTACATATTTAATATTGGATCATTCTTGCAGGTATACTGTGATGCTACCATA GCCTTCCCATTGTTGGTTGCAGAAACATTTGCCACAAAGAGAGACCAAACCTGTGAGTCTAAGACTTAAGA ACTGACTGGTCGTTTTGGCCATGGATTCTTAAAGATCGTTGCTTTTTGATTTTACACTGGAGTGACCATAT AACACTCCACATTGATGTGGCTGTGACGCGAATTGTCTTCTTGCGAATTGTACTTTAGTTTCTCCAACCT AAAATGATTTGCAGATTGTGTTTTCGTTTAAAACACAAGAGTCTTGTAGTCAATAATCCTTTGCCTTATAA AATTATTCAGTTCCAACAACACATTGTGATTCTGTGACAAGTCTCCCGTTGCCTATGTTCACTTCTCTGCG

FIG.2A



AEDCSEEEKNPSFRESVKCKIFLGFTSNLVSSGVRDTIRYLVQHHMVDVIVTTTGGVEEDLIKCLAPTFKGDFSLPGAYLRSK GLNRIGNLLVPNDNYCKFEDWIIPIFDEMLKEQKEENVLWTPSKLLARLGKEINNESSYLYWAYKMNIPVFCPGLTDGSLGDM LYFHSFRTSGLIIDVVQDIRAMNGEAVHANPKKTGMIILGGGLPKHHICNANMMRNGADYAVFINTGQEFDGSDSGARPDEAY MEDDRVFSSVHSTVFKESESLEGKCDKIEGYDFNQGVDYPKLMRSMLTTGFQASNLGEAIDVVNQMFEFVLKLDWRLADETTV SWGKIRGSAKTVKVCFLISSHPNLYLTQWF

FIG.2B

GTCAAAGGGATTGAACCGAATTGGGAATTTGCTGGTTCCTAATGATAACTACTGCAAGTTTGAGGATTGGATCATTCCCA ICTITGACGAGATGTTGAAGGAACAGAAAGAAGAAGAATGTGTTGTGGACTCCTTCTAAACTGTTAGCACGGCTGGGAAAA GAAATCAACAATGAGAGTTCATACCTTTATTGGGCATACAAGATGAATATTCCAGTATTCTGCCCAGGGTTAACAGATGG CTCTCTTAGGGATATGCTGTATTTCACTCTTTTCGTACCTCTGGCCTCATCGATGTAGTACAAGATATCAGAGCTA IGAACGGCGAAGCTGTCCATGCAAATCCTAAAAAGACAGGGATGATAATCCTTGGAGGGGGGCTTGCCAAAGCACCACATA GTAATGCCAATATGATGCGCAATGGTGCAGATTACGCTGTATTTATAAACACCGGGCAAGAATTTGATGGGAGCGACTC GGGTGCACGCCCTGATGAAGC

-1G.2C

GGVEEDLIKCLAPTFKGDFSLPGAYLRSKGLNŘÍGNLLVPNDNYCKFEDWIIPIFDEMLKEQKEENVLWTPSKLLARLGKEIN NESSYLYWAYKMNIPVFCPGLTDGSLRDMLYFHSFRTSGLIIDVVQDIRAMNGEAVHANPKKTGMIILGGGLPKHHICNANMM RNGADYAVFINTGQEFDGSDSGARPDE

FIG.2D

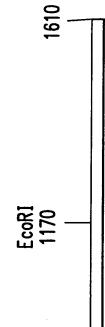




Multiple DHS Sequence Alignments of Human, Arabidopsis, Tomato, Yeast, Neurospora (Fungi), and Methanococcus (Archaeobacteria)

	5/4	415 FMH EKNED IKK VKN	
		02112777	2 401 T FADYNDAFMH WF F FA. SGKPIKK F FA. KEDGL
100 ADETTVALO SHELT FOR SHELT FOR RECHIDE FOR	200 NDNYCKFEDW NDNYCKFEDW NDNYCKFEW NDNYCKFEW NSNYCAFEDW NENYTAFEEY	300 Perv Physical I	400 SSHOWNLTQ IAFFILWAET TVUBLIWAET AAFFIWANT
IEKKI EFVLK DWR LDWR RSW RAY RAY	INRIGNLLVF LNRIGNLLVF NNRIGNLLVF NNRIGNLLVF INRIGNIFVF	THESTAN YELSERTS YELSERRES YELSERRES YELSERRES YELSERRES YELSERRES YELSERRES	CHVKV, CPL I KTVKV, HCDAT SVKL FADVT DAVKMMEAT DOVE I AGDAT
WAY SNDKEF WILDS IN THE WAY SNDKEF	PG PR RSK COMPANY COMP	1031604 1031604 1031604 1031604 1031604	VSWCK LINDA VSWCK LIKESA VSWCK LIKEGA VSWCK LIKAEA VSWCK LIKAEA VSWCK LIGAKA
ASNICERIDO ASNICERIDO ASSICIACEI ASSICERE EDDISLEEI	APTINGERS APTINGERS APTINGERA APTINGERA APTINGERA	AN IPVISTA NA IPVISTA	SDSGARPDEA SDSGARPDEA SDSGARPDEA SDAGARPDEA SLAGSRPDEA SLAGSRPDEA
51 LEAFGIIGH MRSVLTIGH IKSVVSTGFC IEAWKTVGFC LQGWAHVGFC IEGIAIE(PW	151 XVEDLIKO XVEDLIKO XVEDLIKO XVEDLIKO XVEDLIKO	251 NPESVYYWAO DETSYLYWAY DESSYLYWAY DESSYLYWAY CERSVYYWAW	351 MINICOFFDE FINICOFFDE FINICOFFDE MINICOFFDE YVTJALPADE
50 GWDYPK GWDYPK GWNYAEL EATNMRATD KGRP ITVDD	NWDVIVIIA HWDVIVIIA WWDWWTIA WYDAWTSA CHVSAIVTIA KTILIIVIIA	250 MIAKLGKEIN VIARLGKEIN MIDRFGKEIN WIFFLGKEIN	350 WINNEALYAV WINNEALYAV WINNEALYAV MINNEALYAV MINNEALYAV
VRGYDENR TEGYDEN G TEGYDEN K VOGTIDYSK.P VEELDENK.F AKPYGCKRDP	KATINIKYLVOF RDIVRYLVOF FATIRYLVOF ROLLERYLVOF RETIVNIV	TEGVKWIPSK EENVLWIPSK NEKVLWIPSK DSPI.WTPSK ENEINWTPSK ASEFCYKLGE	WKH IAWAN LPKH ICNAN LPKH VONAN IMKH IAWAC IPKH IAWAC
SENTER OF SENTER OF SENTER SOFT SENTERS OF S	YISNL ISSGI FTSNL VSSGV YTSNL ISSGV YTSNL ISSGL YTSNI ISSGL	COEYVKKHG ADCLEANODV DE COEYVKKHG ADCLEANOU DE COEYVKKHG ADCLEANO	CIGMITLEGE KTGMITLGGC KTGMITLGGC RAGMITLGGC RAGMITLGGC RAGMITLGGC RAGMITLGGC
조조조종 동요	PLTSTHFIC ESMOCKFIC GYOKTHFIC GYOKTHFIC MADETTVFFIC		NITORITHE K NGEAVHAGIR NSMSWAR Y NITIANGA K NDIAINS K
1 MEGSLEREAP MEDORVF MGEALKYSIM V.SDINEKLP VADNQIP WOIKDNPIR	human QHADLTQSRK opsis SEEEKNPSFK omato SEEERDVAYK yeast HIKKGCPDEE FungiDPT	201 LMTITE OF WEILER OF WE	301 LEIMEDERLI TEIMEDIRAN TEIMEDIRKI TEIMEDIRKI TEIMEDIRKI TEIMEDIRKI
human Arabidopsis tomato yeast Fungi	human QHADLTQSRF Arabidopsis SEEEKNPSFF tomato SEEERDVAYE yeast HEKGGCPDEE FungiDPT Archaeobacteria IWKHIEEKRK	human Arabidopsis tomato yeast Fungi	human Arabidopsis tomato yeast Fungi Archaeobacteria
Arch	Arch	Arch	Arch

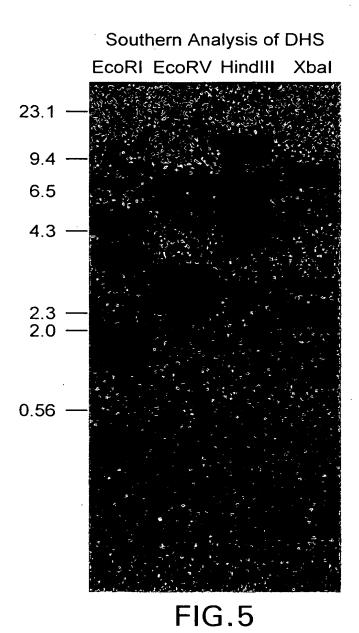




Xbal 121

F16.4







Northern analysis of DHS on tomato flowers

Blossom

and

Bud Senescence

RNA
Northern



NORTHERN ANALYSIS OF DHS ON DEVELOPMENTAL STAGES OF TOMATO FRUIT

BREAKER PINK

RIPE (RED)

NORTHERN BLOT





Northern Analysis of DHS – 2M Sorbitol treated Tomato Leaves

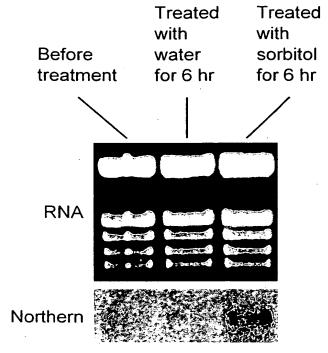


FIG.8



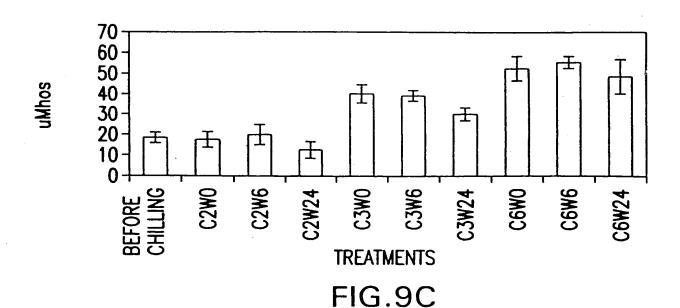
NORTHERN ANALYSIS OF DHS TOMATO LEAF CHILLING EFFECTS

CHILLING 3 DAYS, REWARM 1 DAY, BEFORE CHILLING 2 DAYS CHILLING 2 DAYS CHILLING 6 DAYS CHILLING REWARMING (hr) REWARMING (hr) REWARMING (hr) 0 6 24 24 0 6 0 6 24 **RNA**

FIG.9A

Northern

FIG.9B





Canation DHS cDNA Sequence

GTCATTACAATGCATAGGATCATTGCACATGCTACCTTCCTCATTGCACTTGAGCTTGCCATA GTGTTTTTGAAGTTGTTTTTGATAAGCAGAACCCAGTTGTTTTACACTTTTACCATTGAACTA CTGCAATTCTAAAACTTTGTTTACATTTTAATTCCATCAAAGATTGAGTTCAGCATAGGAAAA AGGATGGAGGATGCTAATCATGATAGTGTGGCATCTGCGCACTCTGCAGCATTCAAAAAGTCG M E D A N H D S V A S A H S A A F K K S GAGAATTTAGAGGGGAAAAGCGTTAAGATTGAGGGTTATGATTTTAATCAAGGTGTAAACTAT ENLEGKSVKIEGYDFNQGVNY TCCAAACTCTTGCAATCTTTCGCTTCTAATGGGTTTCAAGCCTCGAATCTTGGAGATGCCATT S K L L Q S F A S N G F Q A S N L G D A I GAAGTAGTTAATCATATGCTAGATTGGAGTCTGGCAGATGAGGCACCTGTGGACGATTGTAGC EVVNHMLDWSLADEAPVDDC GAGGAAGAGAGGGATCCTAAATTCAGAGAATCTGTGAAGTGCAAAGTGTTCTTGGGCTTTACT EEERDPKFRESVKCKVFLGF TCAAATCTTATTTCCTCTGGTGTTCGTGACACAATTCGGTATCTCGTGCAACATCATATGGTT N L I S S G V R D T I R Y L V Q H H M GACGTGATAGTAACGACAACCGGAGGTATAGAAGAAGATCTAATAAAAGGAAGATCCATCAAG D V I V T T T G G I E E D L I K G R S I K TGCCTTGCACCCACTTTCAAAGGCGATTTTGCCTTACCAGGAGCTCAATTACGCTCCAAAGGG L A P T F K G D F A L P G A O L R S K G TTGAATCGAATTGGTAATCTGTTGGTTCCGAATGATAACTACTGTAAATTTGAGGATTGGATC LNRIGNLLVPNDNYCKFEDWI ATTCCAATTTTAGATAAGATGTTGGAAGAGCAAATTTCAGAGAAAATCTTATGGACACCATCG I P I L D K M L E E Q I S E K I L W T P S AAGTTGATTGGTCGATTAGGAAGAGAAATAAACGATGAGAGTTCATACCTTTACTGGGCCTTC K L I G R L G R E I N D E S S Y L Y W A F AAGAACAATATTCCAGTATTTTGCCCAGGTTTAACAGACGGCTCACTCGGAGACATGCTATAT I P V F C P G L T D G S L G D M L Y K N NTTTCATTCTTTTCGCAATCCGGGTTTAATCGTCGATGTTGTGCAAGATATAAGAGCAGTAAAT F H S F R N P G L I V D V V Q D I R A V N GGCGAGGCTGTGCACGCAGCGCCTAGGAAAACAGGCATGATTATACTCGGTGGAGGGTTGCCT G E A V H A A P R K T G M I I L G G G L AAGCACCACATCTGCAACGCAAACATGATGAGAAATGGCGCCGATTATGCTGTTTTCATCAAC KHHICNANMMRNGADYAVFIN ACTGCCGAAGAGTTTGACGGCAGTGATTCTGGTGCTCGCCCCGATGAGGCTATTTCATGGGGC T A E E F D G S D S G A R P D E A I S W G AAAATTAGCGGATCTGCTAAGACTGTGAAGGTGCATTGTGATGCCACGATAGCTTTCCCTCTA KISGSAKTVKVHCDATIAFPŁ CTAGTCGCTGAGACATTTGCAGCAAAAAGAGAAAAAGAGAGGAAGAGCTGTTAAAACTTTTTT LVAETFAAKREKERKSC GATTGTTGAAAAATCTGTGTTATACAAGTCTCGAAATGCATTTTAGTAATTGACTTGATCTTA TCATTTCAATGTGTTATCTTTGAAAATGTTGGTAATGAAACATCTCACCTCTTCTATACAACA GAGAGTACATTTTTGAGGTAAAAATATAGGATTTTTGTGCGATGCAAATGCTGGTTATTCCCT TGAAAAAAAAAAAAAAAAA (1384 bps, not include Poly A tail and 5'end nocoding region.

FIG. 10

373 Amino Acid.)



Northern Analysis of WT AT Aging Leaves

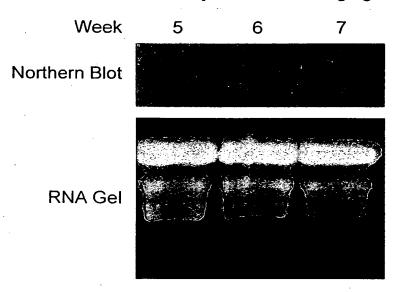


FIG.11



Northern Analysis of Canation Petal (In Situ) DHS

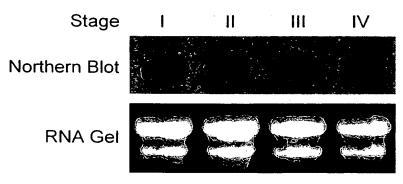


FIG.12





Tomato eif5A

MSDEEH CATTTTGAGTCAAAGGCAGATGCTGGTGCCTCAAAAACTTTCCCACAGCAAGCTGGAACC H F E S K A D A G A S K T F P Q Q A G T ATCCGTAAGAATGGTTACATCGTTATCAAAGGCCGTCCCTGCAAGGTTGTTGAGGTCTCC IRKNGYIVIKGRPCKVV ACTTCAAAAACTGGAAAACACGGACATGCTAAATGTCACTTTGTGGCAATTGACATTTTC T S K T G K H G H A K C H F V A AATGGAAAGAAACTGGAAGATATCGTTCCGTCCTCCCACAATTGTGATGTGCCACATGTT NGKKLEDIVPSSHNCDVPHV AACCGTACCGACTATCAGCTGATTGATATCTCTGAAGATGGTTTTGTCTCACTTCTTACT NRTDYQLIDISEDGFVSLLT GAAAGTGGAAACACCAAGGATGACCTCAGGCTTCCCACCGATGAAAATCTGCTGAAGCAG E S G N T K D D L R L P T D E N L L K O GTTAAAGATGGGTTCCAGGAAGGAAAGGATCTTGTGGTGTCTGTTATGTCTGCGATGGGC V K D G F Q E G K D L V V S V M S A M G GAAGAGCAGATTAACGCCGTTAAGGATGTTGGTACCAAGAAT**TAG**TTATGTCATGGCAGC EEQINAVKDVGTKN ATAATCACTGCCAAAGCTTTAAGACATTATCATATCCTAATGTGGTACTTTGATATCACT CTAGAGAAAGTATTGGCTTTGAGCTTTTGACAGCACAGTTGAACTATGTGAAAATTCTAC

764 bps, not: including Poly(A) tail; 160 amino acids



Carnation-F5A

CTCTTTTACATCAAACAAAAAAATTAGGGTTCTTATTTTAGAGTGAGA

GGCGAAAAATCGAACG**ATG**TCGGACGACGATCACCATTTCGAGTCATCGG M S D D D H H F E S S A CCGACGCCGGAGCATCCAAGACTTACCCTCAACAAGCTGGTACAATCCGC DAGASKTYPQQAGTIR AAGAGCGGTCACATCGTCATCAAAAATCGcCCtTGCAAGGtGGTTGAGGT KSGHIVIKNRPCKVVEV TTCTACCTCCAAGACTGGCAAGCACGGTCATGCCAAATGTCACTTTGTTG STSKTGKHGHAKCHFVA CCATTGACATTTCAACGGCAAGAAGCTGGAAGATATTGTCCCCTCATCC IDIFNGKKLEDIVPSS CACAATTGTGATGTTCCACATGTCAACCGTGTCGACTACCAGCTGCTTGA H N C D V P H V N R V D Y Q L L D TATCACTGAAGATGGCTTTCTTAGTCTGCTGACTGACAGTGGTGACACCA ITEDGFVSLLTDSGDTK AGGATGATCTGAAGCTTCCTGCTGATGAGGCCCTTGTGAAGCAGATGAAG D D L K L P A D E A L V K Q M K EGFEAGKDLILSVMCAM GGGAGAAGAGCAGATCTGCGCCGTCAAGGACGTTAGTGGTGGCAAG**TAG**A G E E Q I C A V K D V S G G K AGCTTTTGATGAATCCAATACTACGCGGTGCAGTTGAAGCAATAGTAATC TCGAGAACATTCTGAACCTTATATGTTGAATTGATGGTGCTTAGTTTGTT TTGGAAATCTCTTTGCAATTAAGTTGTACCAAATCAATGGATGTAATGTC TTGAATTTGTTTTATTTTTGTTTTGATGTTTGCTGtGATTGCATTATGCA AAAAAAAAAA

790 bps, 160 amino acids



Arabidopsis F5A

CTGTTACCAAAAAATCTGTACCGCAAAATCCTCGTCGAAGCTCGCTGCTGCAACC**ATG**TC M S CGACGAGGAGCATCACTTTGAGTCCAGTGACGCCGGAGCGTCCAAAACCTACCCTCAACA DEEHHFESSDAGASKTYPQQ AGCTGGAACCATCCGTAAGAATGGTTACATCGTCATCAAAAATCGTCCCTGCAAGGTTGT A G T I R K N G Y I V I K N R P C K V V TGAGGTTTCAACCTCGAAGACTGGCAAGCATGGTCATGCTAAATGTCATTTTGTAGCTAT EVSTSKTGKHGHAKCHFVAI TGATATCTTCACCAGCAAGAAACTCGAAGATATTGTTCCTTCTTCCCACAATTGTGATGT DIFTSKKLEDIVPSSHNCDV TCCTCATGTCAACCGTACTGATTATCAGCTGATTGACATTTCTGAAGATGGATATGTCAG HVNRTDYQLIDISEDG TTTGTTGACTGATAACGGTAGTACCAAGGATGACCTTAAGCTCCCTAATGATGACACTCT LLTDNGSTKDDLKLPNDDTL GCTCCAACAGATCAAGAGT**GGGTTTGATGATGGAAAAGA**TCTAGTGGTGAGTGTAATGTC LQQIKSGFDDGKDLVVSV AGCTATGGGAGAGGAACAGATCAATGCTCTTAAGGACATCGGTCCCAAG**TGA**GACTAACA AMGEEQINALKDIGPK AAGCCTCCCCTTTGTTATGAGATTCTTCTTCTTCTGTAGGCTTCCATTACTCGTCGGAGA TTATCTTGTTTTTGGGTTACTCCTATTTTGGATATTTAAACTTTTGTTAATAATGCCATC TTCTTCAACCTTTTCCTTCTAGATGGTTTTTATACTTCTTCT

754 bps, not including Poly(A) tail; 158 amino acids



Northern Analysis of WT AT DHS and F5A

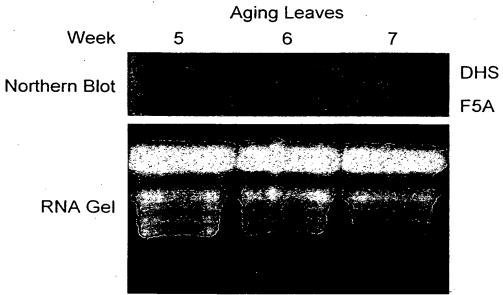


FIG.16



Northern Analysis of Ripening Tomato Fruit

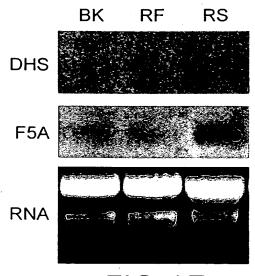


FIG.17



Northern Analysis of sorbitol-treated tomato leaves

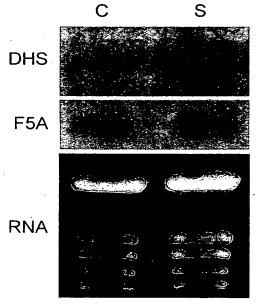


FIG.18



Northern Analysis of Tomato Flowers

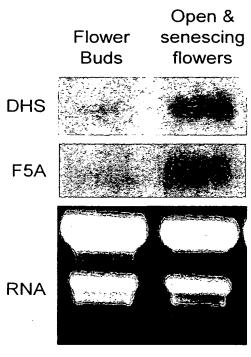


FIG.19



Northern Analysis of chill-injured tomato leaves

Chilling (5°C) treated 6 days

Before rewarming (hr)
chilling 0 6 24

DHS

F5A

RNA

FIG.20

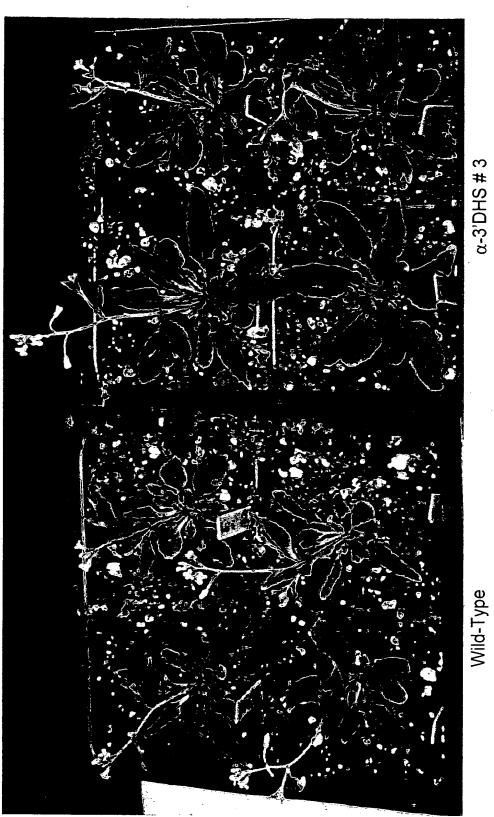


3.1 Weeks

FIG.21

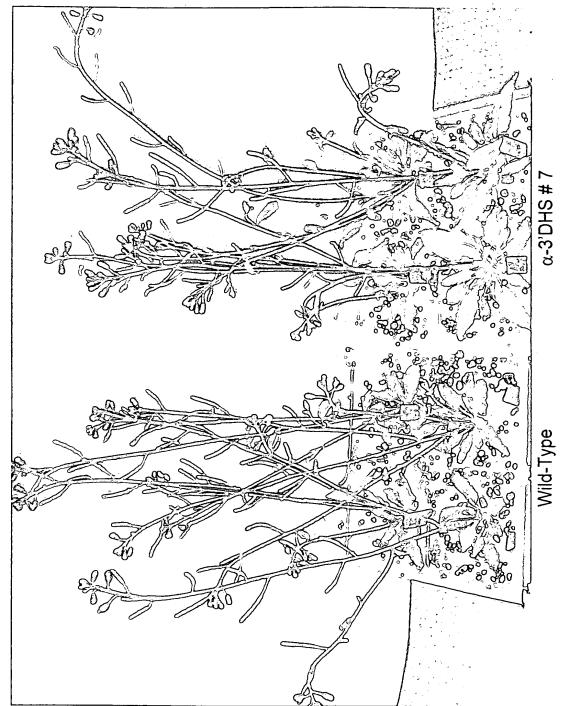


4.6 Weeks



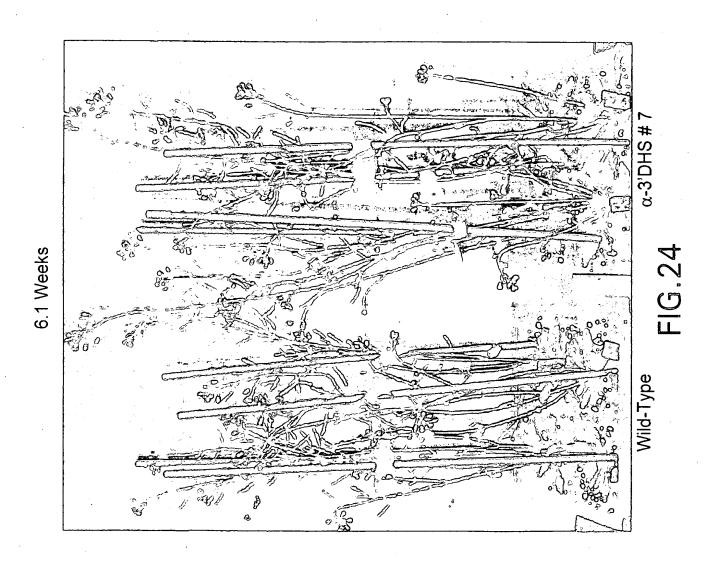
 α -3'DHS # 3





5.6 Weeks







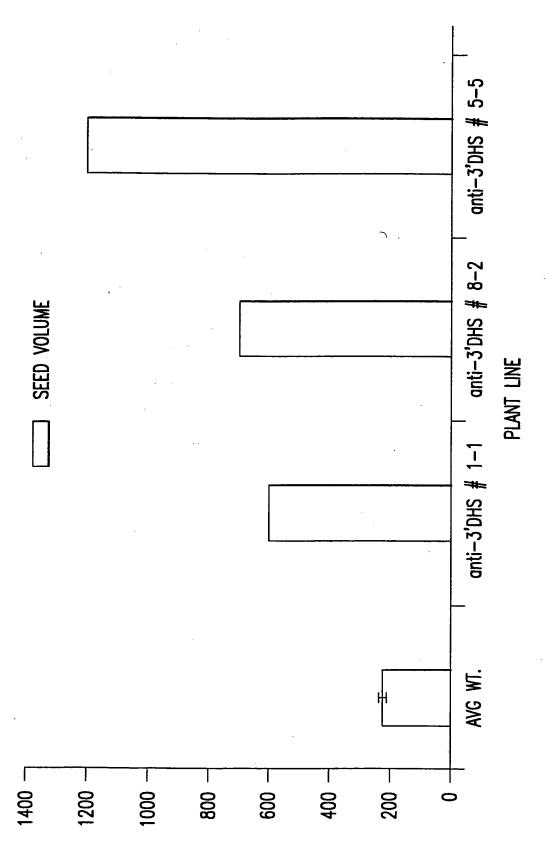


FIG.25





18 Days



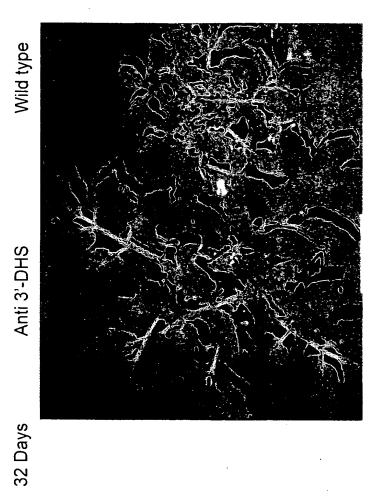


FIG.27

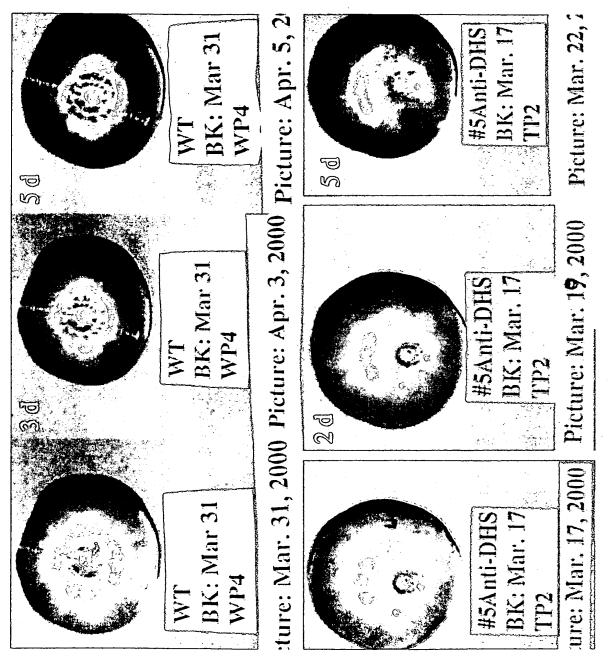


FIG.28



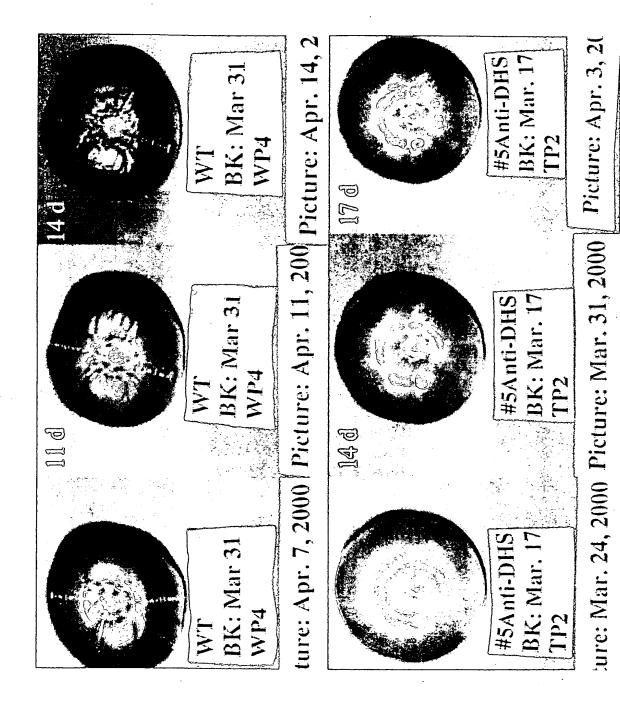


FIG. 29



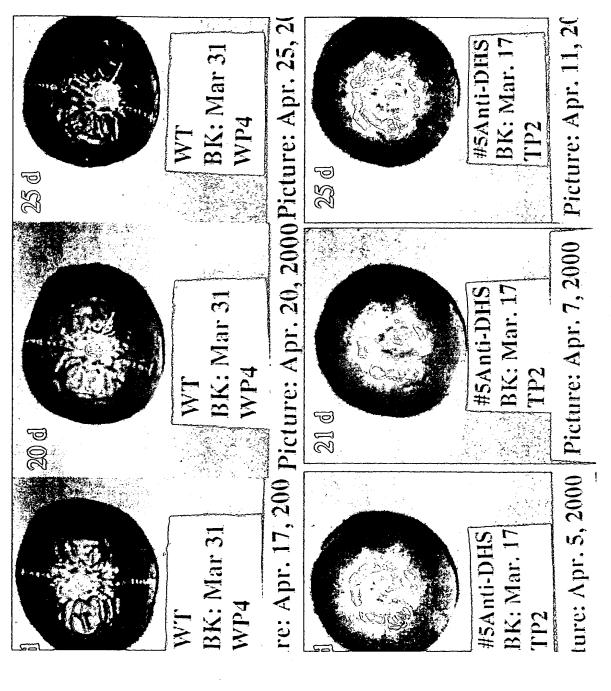


FIG.30



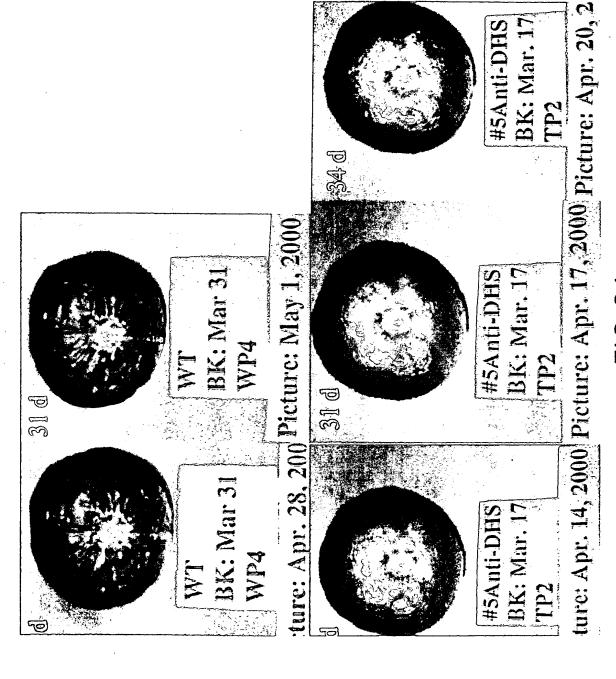


FIG.31



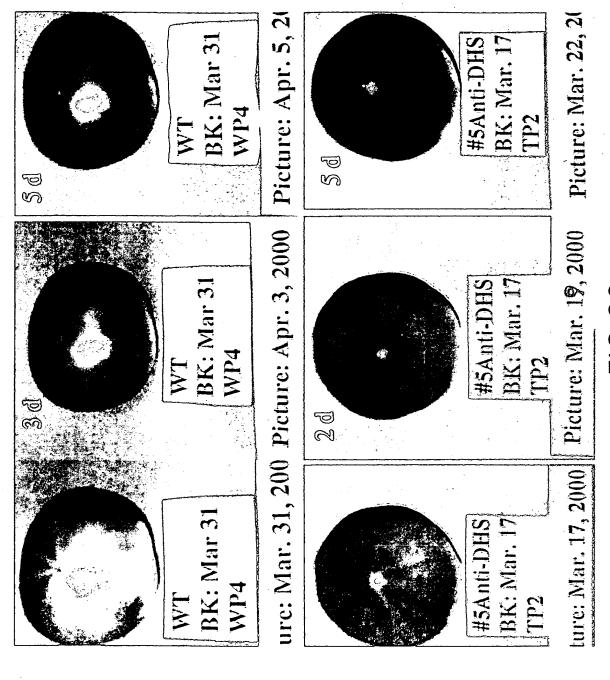


FIG.32



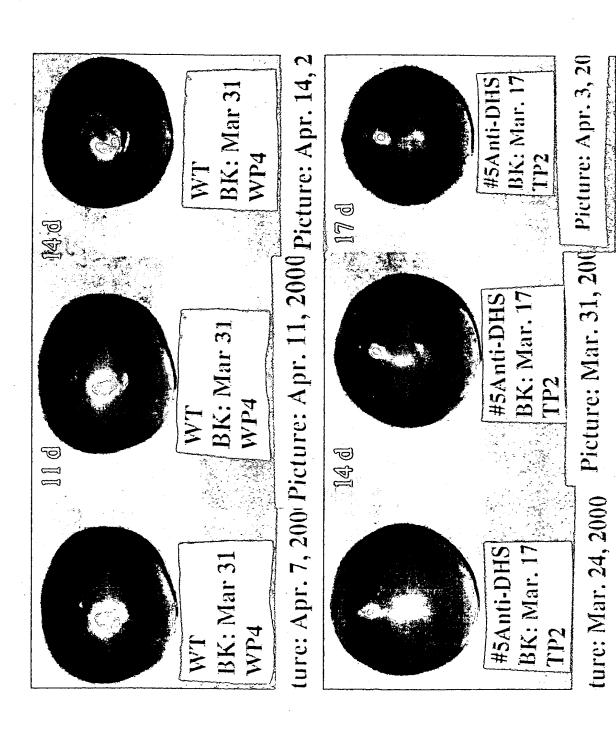


FIG.33



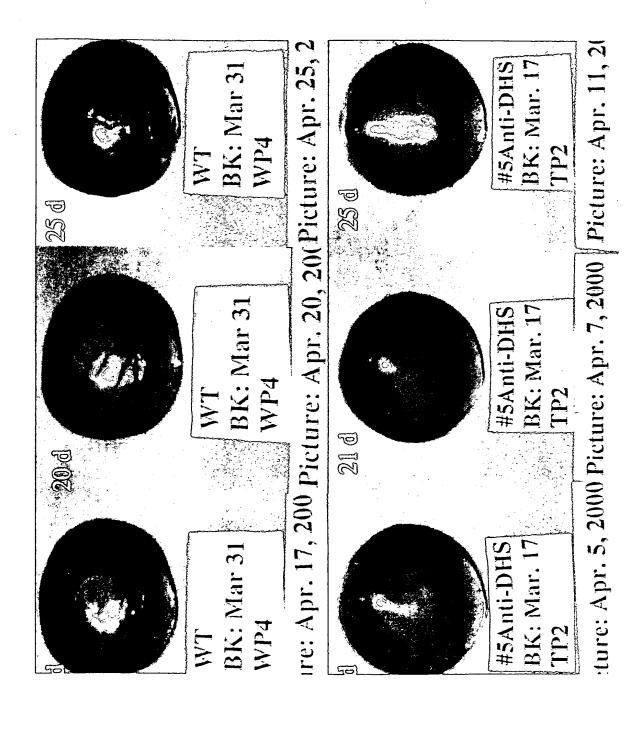


FIG.34



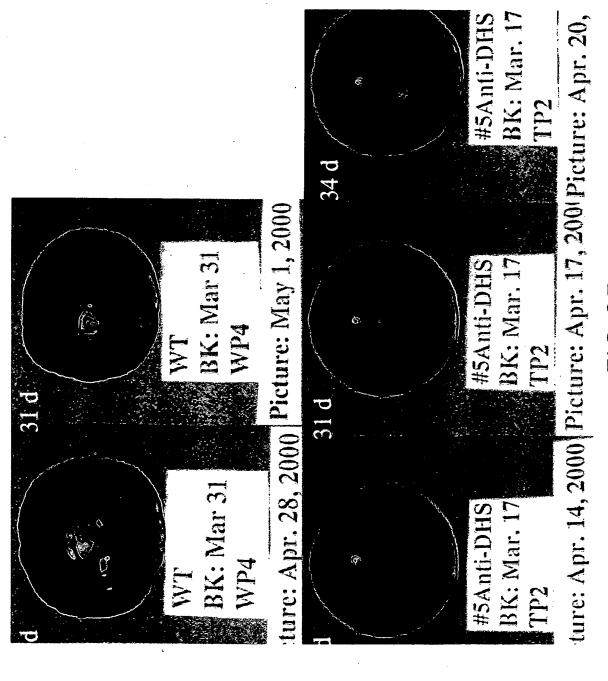


FIG.35



Arabidopsis 3'-end DHS for antisense

Nucleotide and derived amino acid sequence
TGCACGCCCTGATGAAGCTGTGTCTTGGGGTAAAATTAGGGGTTCTGCTAAAACCGTTAAGGTCTGCTTTT
A R P D E A V S W G K I R G S A K T V K V C F

TAATTTCTTCACATCCTAATTTATATCTCACTCAGTGGTTTTGAGTACATATTTAATATTGGATCATTCTT L I S S H P N L Y L T Q W F

Nucleotide sequence

ARPDEAVSWGKIRGSAKTVKVCFLISSHPNLYLTQWF



Tomato 3'-end-Deoxyhupsine synthase used for antisense

GGTGCTCGTCCTGATGAAGCTGTATCATGGGGAAAGATACGTGGTGGTGCCAAGACTGTGAAGGTGCATTGTGATGCAAC ェ <u>></u> ¥ ഗ G Nucleotide and derived amino acid sequence G K I S CATTGCATTTCCCATATTAGTAGCTGAGACATTTGCAGCTAAGAGTAAGGAATTCTCCCAGATAAGGTGCCAAGTTTGAA S ц. ш FAAK بىا CATTGAGGAAGCTGTCCTTCCGACCACATATGAATTGCTAGCTTTTGAAGCCAACTTGCTAGTGTGCAGCACCATTTA TTTCTCCCCTTCACACCATGTTATTTAGTTCTCTTCCTCCAAAGTGAAGAGCTTAGATGTTCATAGGTTTTTGAAT

Nucleotide sequence

GGTGCTCGTCCTGATGAAGCTGTATCATGGGGAAAGATACGTGGTGGTGCCAAGACTGTGAAGGTGCATTGTGATGCAAC CATTGCATTTCCCATATTAGTAGCTGAGACATTTGCAGCTAAGAGTAAGGAATTC

ATCCTGTATGGTTCAAATTAATTATTTTCTCCCCTTCACACCATGTTATTTAGTTCTCTTCCTCTTCGAAAGTGAAGAG TCCCAGATAAGGTGCCAAGTTTGAACATTGAGGAAGCTGTCCTTCCGACCACACATATGAATTGCTAGCTTTTGAAGCCA <u> ACTTGCTAGTGTGCAGCACCATTTATTCTGCAAAACTGACTAGAGAGCAGGGTATATTCCTCTACCCCGAGTTAGACGAC</u> CTTAGATGTTCATAGGTTTTGAATTATGTTGGAGGTTGGTGATAACTGACTAGTCCTCTTACCATATAGATAATGTATCC TGTACTATGAGATTTTGGGTGTTTTGATACCAAGGAAAAATGTTTTGGAAAACAATTGGATTTTAATTTAAAAA AAATTGNTTAAAAAAAAAAAAAAAA



600 bp Arabidopsis Deoxyhypusine Synthase Probe

Primer1 (underlined)

<u>uc</u>	<u>, i uu</u>	<u>ııuı</u>	<u> Lu</u>	<u>iuur</u>	<u> YUY</u>	<u> </u>	CAI	~~~	WIC	スししょ	I UC	MUU	IMC	MII	170	WUC	1 I G/	1111	CIL	. 1 (-) 1	AUL	, ו שט	MUL
										Т	TAT	TTA	AG										
G	G	٧	Ε	Ε	D	L	I	K	С	L	Α	Р	T	F	Κ	G	D	F	S	L	Р	G	Α
										Υ	L	R					,						
GT	CAA	AGG	GAT	TGA	ACC	GAA	TTG	GGA	TTA	TGC	TGG	TTC	CTA	ATG	ATA	ACT	ACT	GCA	AGT	TTG	AGG	TTA	GGA
TCATTCCCA																							
S	Κ	G	L	N	R	Ι	G	N	L	L	٧	P.	N	D	N	Υ	С	Κ	F	F	D	W	I
											I	P				·	•	••	•	_	_	••	
TCTTTGACGAGATGTTGAAGGAACAGAAAGAAGAAGAAGAATGTGTTGTGGACTCCTTCTAAACTGTTAGCACGG																							
											TGG									.0.0			-
Ι	F	Ð	Ε	М	L	Κ	Ε	0	K	F				L	W	Т	P	S	K	ı	1	Α	R
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GA	ΑΑΤ	CAA	CAA	TGA	GAG	TTC	ΔΤΔ	CCT	TΤΔ	TTG				GAT	GΔΔ	ΤΔΤ	ፐርር	ΔΩΤ	ΔΤΤ	стс	የ	AGG	GTT
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Ε	Ι	N	N	Ε	S	S	Υ	L	Υ		Α			М	N	I	Р	٧	F	С	F	G	1
						_	•	_	•	т		-		••	••	-	•	•	•	Ū	•	•	_
СТ	СТС	TTA	GGG	АТА	TGC	TGT	ΆΤΤ	TTC	ACT	стт	_	_		CTG	GCC	TCA	TCA	TCG	ΔΤΩ	TΔG	ΤΔΓ	AAG	ΔΤΔ
•		``									CAG			0.0			0,		, (1 G	11710	1710	, u .a	, , .
										•	<i>0,</i> (0	, ,,,,											
S	ı	R	D	M	ı	Υ	F	Н	S	F	R	т	ς	G	L	т	I	D	٧	٧	0	D	Ι.
	_	•		••	_		'	• • •	J	•	R	A	J	u	_	_	1	U	٧	٧	ų	U	1
TG	ΔΔ	ርርՐ	GΔΔ	GCT	GTC	САТ	CC V	۸۸۳	ጉጉጉ	·			CCC	ATC	ΔΤΛ	۸ΤΩ	CTT	CCV	CCC	בבר	TTC	CCA	۸ ۸ C
1 4	, v (C	uuo	CI V (uc i	uic		uch	ורערו	CCI		ACC			ли		и	CII	uun	uuu	uuc	110	CCA	AAG
М	N	G	Ε	Α	V	Н	Α	N	Р	K		T		М	I	Ţ	ı	G	G	G	ı	P	K
11	14	u	_	^	٧	11	^	IX	Γ.	Н		•	u	ľ	1	1	L	u	u	u	L	Г	N
TC	ΤΛΛ'	TCC	C A A	TAT	CAT		~ A A	TCC	TCC		- •	_	тст	' A T' T'	T A T		C A C	ccc	CC A	A C A	A T T	TC 4	TCC
ıu	TGTAATGCCAATATGATGCGCAATGGTGCAGATTACGCTGTATTTATAAACACCGGGCAAGAATTTGATGG GAGCGACTC																						
_	N.I		A.1	14	1.4	_		_		_ u	AUL V	JAL.	16	_	-		_	_	_	_	_		_

S D S GGGTGCACGCCCTGATGAAGC

GARPDE

Primer 2 (underlined)



483 bp Carnation Deoxyhypusine Synthase Probe

GAAGATCCATCAAGTGCCTTGCACCCACTTTCAAAGGCGATTTTGCCTTACCAGGAGCTCAATTACGCTCC AAAGGGT

R R S I K C L A P T F K G D F A L P G A Q L R S K G

TGAATCGAATTGGTAATCTGTTCCGAATGATAACTACTGTAAATTTGAGGATTGGATCATTCCAATT
TTAGATA

L N R I G N L L V P N D N Y C K F E D W I I P I L D

AGATGTTGGAAGAGCAAATTTCAGAGAAAATCTTATGGACACCATCGAAGTTGATTGGTCGATTAGGAAGA GAAATAA

K M L E E Q I S E K I L W T P S K L I G R L G R E I

ACGATGAGAGTTCATACCTTTACTGGGCCTTCAAGAACAATATTCCAGTATTTTGCCCAGGTTTAACAGAC
GGCTCAC

N D E S S Y L Y W A F K N N I P V F C P G L T D G S

TCGGAGACATGCTATATTTTCATTCTTTTCGCAATCCGGGTTTAATCATCGATGTTGTGCAAGATATAAGA GCAGTAA

L G D M L Y F H S F R N P G L I I D V V Q D I R A V

ATGGCGAGGCTGTGCACGCAGCGCCTAGGAAAACAGGCATGATTATACTCGGTGGAGGGTTGCCTAAGCAC CACATCT

N G E A V H A A P R K T G M I I L G G G L P K H H I

> GCAACGCAAACATGATGAGAAATGGCGCCGATTATGCTGTTTTCATCAACACCG C N A N M M R N G A D Y A V F I N T

A full-length cDNA clone was obtained by screening a carnation senescing petal cDNA library with this probe.



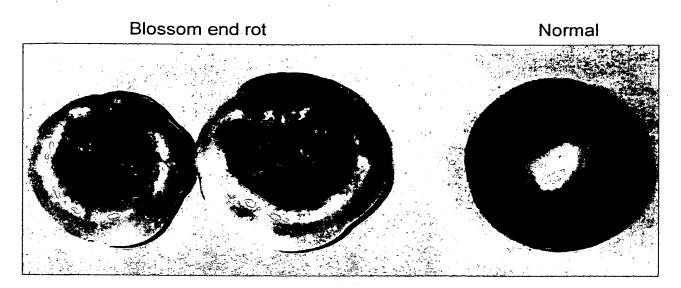


FIG.40A

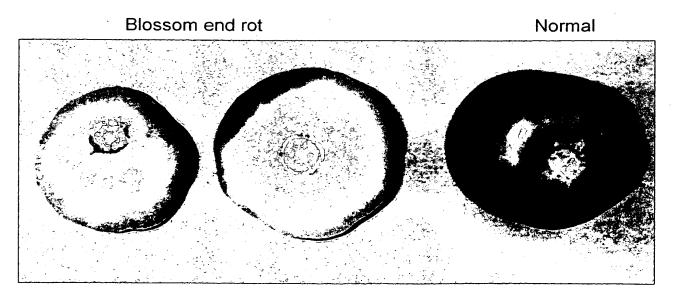


FIG.40B